

PATENT SPECIFICATION

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(54) ENZYME-ACTIVATED OXIDATIVE PROCESS FOR COLOURING HAIR

(71) We, THE PROCTER & GAMBLE COMPANY, a corporation organised under the laws of the State of Ohio, United States of America, of 301 East Sixth Street, Cincinnati, Ohio 45202, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—  
This invention relates to an enzyme activated process for coloring hair.  
Pre-formed dyes for coloring hair have not hitherto been widely accepted due to their general failure to impart to hair colors which are sufficiently imitative of natural hair colors. Products which contain reactants such as H<sub>2</sub>O<sub>2</sub> and an oxidation dye precursor (i.e., a precursor which forms dyes *in situ* on the hair through an oxidative process) do produce colors which are closely imitative of natural hair color and have obtained reasonable commercial success. These latter products, however, because of the severe oxidizing conditions (i.e., performing the oxidation at H<sub>2</sub>O<sub>2</sub> concentrations of 3.0% or more and a pH of 8.5 or higher for time periods in excess of 20 minutes) and high concentrations of oxidation dye precursors needed to produce the desired coloration, can cause skin irritation and sensitization as well as hair damage to some users. Further, the ammonium hydroxide which is generally used to maintain the high pH in these products has an offensive odor.  
U.S. Patent 3,251,742, issued May 17, 1966, discloses an enzyme-based oxidative process for coloring hair at substantially neutral pH, wherein oxygen from the atmosphere is the oxidizing agent, the enzyme is an oxidase (e.g., tyrosinase or laccase) and the dye precursor is a combination of an aromatic polyhydric compound and an aromatic amine.

The present invention is based on the surprising observation that an oxidative coloring system based on oxygen and one of the particular oxidases specified herein does not require the presence of a combination of aromatic polyhydric and aromatic amine precursors. Either type of precursor can be used alone in the present invention. This provides more flexibility in selection of oxidation dye precursors and thus more freedom in the choice of colors to be produced.  
An object of this invention is to provide an improved mild oxidative process for coloring hair.  
According to the invention a process for coloring hair comprises contacting the hair with an aqueous solution of from 0.01 ppm to 500 ppm of an oxidase enzyme selected from laccase, lactate oxidase, glucose oxidase, galactose oxidase, L-2 hydroxy acid oxidase, aldehyde oxidase, monoamine oxidase and urate oxidase, and from 0.001% to 6% by weight of an aromatic compound which is a primary oxidation precursor as hereinafter defined, said solution being in contact with oxygen and having pH from 4 to 10, and being free from mixtures of aromatic amines or derivatives thereof with polyhydric phenols or derivatives thereof.  
The oxidase enzymes suitable for use in the process of this invention are listed in Table 1, together with their classification number (according to The Recommendations (1964) of The International Union of Biochemistry on The Nomenclature and Classification of Enzymes) and their most common sources. The nomenclature system referred to above and a list of known enzymes classified according to it are set forth in "Comprehensive Biochemistry", Florkin and Stortz, Volume 13, Enzyme Nomenclature (1965), published by Elsevier Publishing Company, New York, N.Y.

TABLE I

Enzyme	Classification Number	Common Source
Lactate oxidase	1.1.3.2	<i>Mycobacterium phlei</i>
Glucose oxidase	1.1.3.4.	<i>Aspergillus niger</i>
Galactose oxidase	1.1.3.9	<i>Dactylium dendroides</i>
L—2 hydroxyacid oxidase	1.1.3.a	Hog renal cortex
Aldehyde oxidase	1.2.3.1	Rabbit liver
Monoamine oxidase	1.4.3.4	Beef plasma
Urate oxidase	1.7.3.3	Hog liver
Laccase	1.10.3.2	<i>Polyporus versicolor</i>

5 The enzymes herein can be used in their pure crystalline form, which is obtained by isolating them from other materials present during their preparation, or they can be used in a diluted form, where the enzyme is present in a composition together with these materials and/or added inert diluents.

10 Commercially available enzyme preparations normally contain the enzyme in combination with inert diluent and carrier materials such as carbohydrates, agglutinating proteins, inorganic salts, for example sodium sulfate and calcium sulfate. In such preparations, the enzyme constitutes a minor component and comprises from 1% to 50% by weight of the preparation. The remaining 50% to 99% comprises the hereinbefore described diluents and carriers. The commercially available enzyme-containing preparations are preferred as sources of enzyme herein because they are more readily available than pure crystalline enzyme and provide known, pre-determined and desirable levels of enzyme activity.

25 In the coloring process herein, the oxidase enzyme is used at concentrations within the range from 0.01 ppm to 500 ppm, and preferably from 0.05 ppm to 100 ppm in the coloring solution. These levels are based on weight of pure enzyme. If a commercial enzyme preparation is used, wherein the enzyme is combined with diluents and carriers, as hereinbefore described, the concentration of enzyme preparation must be proportionately higher, in order to achieve the required concentration of pure enzyme. The amount of pure enzyme present in such compositions can be readily determined by known assay methods.

For the purposes of this invention, oxidation

dye precursors are classified as "primary oxidation dye precursors" and "secondary dye precursors". 40

Primary oxidation dye precursors include those aromatic nonionic compounds which, on oxidation, form oligomers or polymers having extended conjugated systems of electrons in their molecular structures and which oligomers or polymers, because of the new electronic structure, exhibit a shift in their visible absorption spectra and appear coloured. For example, primary oxidation dye precursors include such materials as various aromatic amines or phenols which, on oxidation, form a series of conjugated imine or quinonoid dimers, trimers and higher oligomers ranging in color from green to black. Compounds such as *p*-phenylene diamine which have two functional groups and are capable of oxidative polymerization to yield higher molecular weight coloured materials having extended conjugated electron systems (the so-called "Bandrowski's Base" type of dye compound), are also primary oxidation polymers. The primary oxidation dye precursors which are essential for use in the process of this invention are defined herein as those aromatic compounds which produce color formation in the following test, which is carried out at room temperature (ca. 18—28° C). 45 50 55 60 65

#### PRIMARY OXIDATION DYE PRECURSOR TEST 70

Approximately 10 ml. of aqueous buffer (pH 5 to 8) is mixed with 0.1 to 1.0 ml. of a 1% (by weight) aqueous or alcoholic solution of the potential dye precursor. To this mixture is added laccase in an amount such 75

that the final mixture contains 0.01 to 100 ppm of laccase (based on weight of pure enzyme). The mixture is allowed to stand exposed to the atmosphere. A suitable primary oxidation dye precursor will give color formation within 20 minutes. Some dye precursors, because of their own color, impart a pale color to the solution before addition of enzyme. Color formation, in this test, refers to the color change which occurs after the addition of enzyme.

Secondary oxidation dye precursors or color modifiers may optionally be employed together with the primary oxidation dye precursors in the process of this invention, and are believed to interpose themselves in the coloured polymers during their formation and to cause shifts in the electronic spectra thereof, thereby resulting in changes in colour shade and/or colour intensity. They include aromatic compounds such as amines and phenols which do not produce color formation in the above "PRIMARY OXIDATION DYE PRECURSOR TEST", but which modify the color shade or intensity of color developed by the corresponding type of primary oxidation dye precursor; i.e. amine (and derivatives thereof) secondary oxidation dye precursors modify the color, shade or intensity of color produced by amine primary oxidation dye precursors and derivatives thereof which phenolic (and derivatives thereof) secondary oxidation dye precursors modify the shade or intensity of color produced by phenolic primary dye precursors and derivatives thereof. The secondary oxidation dye precursors which are suitable for use with the primary oxidation dye precursors in the process of this invention are defined as those aromatic compounds which are not primary oxidative dye precursors, but which modify the color, shade or intensity of color produced by the corresponding type of primary oxidation dye precursor in the following test which is carried out at room temperature (ca. 18—28° C).

#### SECONDARY OXIDATION DYE PRECURSOR TEST

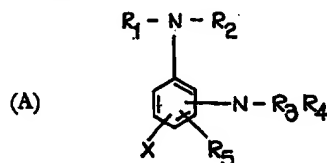
Two solutions are prepared as follows: approximately 10 ml. of aqueous buffer (pH 5 to 8) is mixed with 0.1 to 1.0 ml. of a 1.0% (by weight) aqueous or alcoholic solution of primary oxidation dye precursor. 0.1 to 1 ml. of a 1% (by weight) aqueous or alcoholic solution of the potential secondary oxidation dye precursor is added to one of the solutions, and then laccase is added to each solution in an amount such that the final enzyme concentration is from 0.01 to 100 ppm (based on weight of pure enzyme). The solutions are left to stand exposed to air for 20 minutes to permit development of color. A suitable secondary oxidation dye precursor will cause the second

solution to differ from the first solution in color, shade or intensity of color.

It is to be understood that the oxidase enzymes disclosed herein are suitable for use (in conjunction with oxygen as detailed herein) with all manner of primary and secondary oxidation dye precursors, provided that primary and secondary oxidation dye precursors, which are polyhydric phenolics or derivatives thereof, are not used in combination with primary and secondary oxidation dye precursors which are diamines or derivatives thereof, and primary and secondary oxidation dye precursors which are amino compounds or derivatives thereof are not used in combination with primary and secondary oxidative dye precursors which are polyhydric phenols or derivatives thereof. A representative list of oxidation dye precursors suitable for use herein is found in Sagarin, "Cosmetic Science and Technology", Interscience, pages 504 and 508, and the dye precursors detailed below are by way of example only. Additional oxidation dye precursors useful herein are described in French Application 1,318,072 and French Addition 90,633, January 19, 1968, (Schwarzkopf); British Patent 1,127,080 (Kalopissis and Bagaut); and Netherlands Application 6,609,833, February 6, 1967, (Therachemie Chemisch Therapeutische G.m.b.H.). Pyridine, quinoline, and isoquinoline oxidation dye precursors such as those disclosed by Bergwein, *Reichst. Aromen, Koerperpflegem.* 17 (14) 136—8 (1967), are also suitable for use herein.

The aromatic diamines and polyhydric phenols and derivatives thereof, described above as primary oxidation dye precursors, can also have additional substituents on the aromatic ring, for example halogen, aldehyde, carboxylic acid, nitro, sulphonic acid and substituted and unsubstituted hydrocarbon groups, as well as additional substituents on the amino nitrogen and on the phenolic oxygen, for example substituted and unsubstituted alkyl and aryl groups. The aromatic diamines and derivatives thereof, however, should not contain hydroxyl groups and polyhydric phenols and derivatives thereof should not contain amino groups, inasmuch as the dye precursors herein must be free from mixtures of aromatic amines, or derivatives thereof, with polyhydric phenols, or derivatives thereof.

Examples of aromatic diamines and derivatives thereof and polyhydric phenols and derivatives thereof, respectively, are compounds having the general formulas A and B, below:



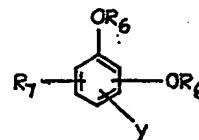
wherein X is hydrogen, halogen, (e.g., fluorine, chlorine, bromine or iodine), nitro, amino,



- COOM or —SO<sub>3</sub>M (where M is hydrogen or an alkali metal or alkaline earth metal, ammonium, or substituted ammonium wherein one or more hydrogens on the ammonium ion is replaced with a 1 to 3 carbon atom alkyl radical), and R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are the same or different from each other and each represents a hydrogen atom, a C<sub>1</sub> to C<sub>4</sub> alkyl or alkenyl or a C<sub>6</sub> to C<sub>8</sub> aryl, alkaryl or aralkyl, and R<sub>5</sub> is hydrogen, a C<sub>1</sub> to C<sub>4</sub> alkyl or alkenyl (including substituted alkyl or alkenyl wherein the substituents are selected from those designated as X, above), or a C<sub>6</sub> to C<sub>8</sub> aryl, alkaryl or aralkyl (including substituted aryl, alkaryl or aralkyl group wherein the substituents are selected from those defined as X, as above). Specific examples of formula (A) compounds are:

- o* - phenylenediamine
- m* - phenylenediamine
- p* - phenylenediamine
- 2 - chloro - *p* - phenylenediamine
- 2 - iodo - *p* - phenylenediamine
- 4 - nitro - *o* - phenylenediamine
- 2 - nitro - *p* - phenylenediamine
- 1,3,5 - triaminobenzene
- 2,4 - diaminobenzoic acid
- sodium 2,4 - diaminobenzoate
- calcium di - 2,4 - diaminobenzoate
- ammonium 2,4 - diaminobenzoate
- trimethylammonium 2,4 - diaminobenzoate
- 2,4 - diaminobenzaldehyde
- 2,4 - diaminobenzenesulfonic acid
- potassium 2,4 - diaminobenzenesulfonate
- N,N - diisopropyl - *p* - phenylenediamine
- N,N - dimethyl - *p* - phenylenediamine
- N - methyl - N' - (2 - propenyl) - *p* - phenylenediamine
- N - phenyl - *p* - phenylenediamine
- N - phenyl - N - benzyl - *p* - phenylenediamine
- N - ethyl - N' - (3 - ethylphenyl) - *p* - phenylenediamine
- 2,4 - toluenediamine
- 2 - ethyl - *p* - phenylenediamine
- 2 - (2 - bromoethyl) - *p* - phenylenediamine
- 2 - phenyl - *p* - phenylenediamine
- 4 - (2,5 - diaminophenyl)benzaldehyde
- 2 - benzyl - *p* - phenylenediamine
- 2 - (4 - nitrobenzyl) - *p* - phenylenediamine
- 2 - (4 - methylphenyl) - *p* - phenylenediamine
- 2 - (2,5 - diaminophenyl) - 5 - methylbenzoic acid

(B)



wherein Y is hydrogen, halogen (e.g., fluorine, chlorine, bromine or iodine), nitro, hydroxyl,



- COOM', or SO<sub>3</sub>M' (where M' is hydrogen or an alkali metal or alkaline earth metal, wherein R<sub>6</sub> is hydrogen or a C<sub>1</sub> to C<sub>4</sub> alkyl or alkenyl (including substituted alkyl or alkenyl wherein the substituents are selected from those defined as Y, above) and R<sub>7</sub> is hydrogen, a C<sub>1</sub> to C<sub>4</sub> alkyl or alkenyl (or substituted alkyl or alkenyl wherein the substituents are selected from those designated as Y, above) or a C<sub>6</sub> to C<sub>8</sub> aryl, alkaryl or aralkyl group (including substituted aryl, alkaryl or aralkyl wherein the substituents are selected from those designated as Y). Specific examples of formula (B) compounds are:

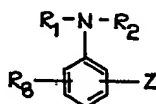
- o* - hydroxyphenol
- m* - hydroxyphenol
- p* - hydroxyphenol
- 4 - methoxyphenol
- 2 - methoxyphenol
- 4 - (2 - chloroethoxy)phenol
- 4 - (2 - nitroethoxy)phenol
- 4 - (2 - hydroxyethoxy)phenol
- (4 - hydroxyphenoxy)acetaldehyde
- (4 - hydroxyphenoxy)acetic acid
- 2 - (4 - hydroxyphenoxy)ethanesulfonic acid
- 4 - (2 - propenoxy)phenol
- 4 - (3 - chloro - 2 - propenoxy)phenol
- 2 - chloro - 4 - hydroxyphenol
- 2 - nitro - 4 - hydroxyphenol
- 1,3,5 - trihydroxybenzene
- 2,4 - dihydroxybenzaldehyde
- 3,4 - dihydroxybenzaldehyde
- 3,4 - dihydroxybenzenesulfonic acid
- 2,4 - dihydroxybenzenesulfonic acid
- 3 - ethyl - 4 - hydroxyphenol
- 3 - (2 - nitroethyl) - 4 - hydroxyphenol
- 3 - (2 - propenyl) - 1 - hydroxyphenol
- 3 - (3 - chloro - 2 - propenyl) - 4 - hydroxyphenol
- 2 - phenyl - 4 - hydroxyphenol
- 2 - (4 - chlorophenyl) - 4 - hydroxyphenol
- 2 - benzyl - 4 - hydroxyphenol
- 2 - (2 - nitrophenyl) - 4 - hydroxyphenol
- 2 - (2 - methylphenyl) - 4 - hydroxyphenol
- 2 - (2 - methyl - 4 - chlorophenyl) - 4 - hydroxyphenol

3 - methoxy - 4 - hydroxy - benzaldehyde  
 2 - methoxy - 4 - (1 - propenyl)phenol  
 4 - hydroxy - 3 - methoxycinnamic acid

- 5 The aromatic amines and phenols and derivatives thereof described above as secondary oxidation dye precursors can also have additional substituents on the aromatic ring, e.g., halogen, aldehyde, carboxylic acid, nitro, sulfonyl and substituted and unsubstituted hydrocarbon groups. The aromatic amines and derivatives thereof, however, should not contain hydroxyl groups and the phenols and derivatives thereof should not contain amino groups.

- 15 Examples of aromatic amines, phenols and derivatives thereof are compounds of the general formulae (C) and (D) below:

(C)



- 20 wherein Z is hydrogen, a C<sub>1</sub> to C<sub>6</sub> alkyl, halogen (e.g., fluorine, chlorine, bromine or iodine) nitro,

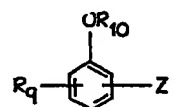


—COOM or —SO<sub>3</sub>M, (where M is hydrogen or an alkali metal or alkaline earth metal, ammonium or substituted ammonium wherein one or more hydrogens on the ammonium ion is replaced by a 1 to 3 carbon atom alkyl radical), wherein R<sub>1</sub> and R<sub>2</sub> are the same or different and each represents hydrogen, a C<sub>1</sub> to C<sub>4</sub> alkyl or alkenyl or a C<sub>6</sub> to C<sub>6</sub> aryl, alkaryl or aralkyl and R<sub>3</sub> is hydrogen, a C<sub>1</sub> to C<sub>4</sub> alkyl or alkenyl (including substituted alkyl or alkenyl wherein the substituents are selected from those designated as Z, above) or a C<sub>6</sub> to C<sub>6</sub> aryl, alkaryl or aralkyl group (including substituted aryl, alkaryl or aralkyl wherein the substituents are selected from those defined as Z, above). Specific examples of formula (C) compounds are:

- 40 aniline  
 p - chloroaniline  
 p - nitroaniline  
 p - aminobenzaldehyde  
 p - aminobenzoic acid  
 45 sodium - p - aminobenzoate  
 lithium - p - aminobenzoate  
 calcium di - p - aminobenzoate  
 ammonium p - aminobenzoate  
 trimethylammonium p - aminobenzoate

- p - aminobenzenesulfonic acid 50  
 potassium p - aminobenzenesulfonate  
 N - methylaniline  
 N - propyl - N - phenylaniline  
 N - methyl - N - 2 - propenylaniline  
 3,4 - dimethylaniline 55  
 N - benzylaniline  
 N - (2 - ethylphenyl)aniline  
 4 - methylaniline (p - toluidine)  
 4 - (2 - bromoethyl)aniline  
 2 - (2 - nitroethyl)aniline 60  
 (4 - aminophenyl)acetaldehyde  
 (4 - aminophenyl)acetic acid  
 4 - (2 - propenyl)aniline  
 4 - (3 - bromo - 2 - propenyl)aniline  
 4 - phenylaniline 65  
 4 - (3 - chlorophenyl)aniline  
 4 - benzylaniline  
 4 - (4 - iodobenzyl)aniline  
 4 - (3 - ethylphenyl)aniline  
 4 - (2 - chloro - 4 - ethylphenyl)aniline 70

(D)



wherein Z' is hydrogen, a C<sub>1</sub> to C<sub>6</sub> alkyl, halogen (e.g., chlorine, fluorine, bromine or iodine), a nitro,



—COOM' or SO<sub>3</sub>M' (where M' is hydrogen or an alkali metal or alkaline earth metal), wherein R<sub>4</sub> is hydrogen, a C<sub>1</sub> to C<sub>4</sub> alkyl or alkenyl (including substituted alkyl or alkenyl wherein the substituents are selected from those designated as Z', above) or a C<sub>6</sub> to C<sub>6</sub> aryl, alkaryl or aralkyl (including substituted aryl, alkaryl or aralkyl wherein the substituents are selected from those designated as Z' above) and R<sub>10</sub> is hydrogen or a C<sub>1</sub> to C<sub>4</sub> alkyl or alkenyl (including substituted alkyl or alkenyl wherein the substituents are selected from those defined as Z in formula (C), above). Specific examples of formula (D) compounds are:

- phenol 90  
 p - chlorophenol  
 p - nitrophenol  
 p - hydroxybenzaldehyde  
 p - hydroxybenzoic acid  
 p - hydroxybenzenesulfonic acid 95  
 ethylphenyl ether  
 2 - chloroethylphenyl ether  
 2 - nitroethylphenyl ether  
 phenoxyacetaldehyde  
 phenoxyacetic acid 100  
 3 - phenoxy - 1 - propene

- 3 - phenoxy - 2 - nitro - 1 - propene
- 3 - phenoxy - 2 - bromo - 1 - propene
- 4 - propylphenol
- 4 - (3 - bromopropyl)phenol
- 5 2 - (2 - nitroethyl)phenol
- (4 - hydroxyphenyl)acetaldehyde
- (4 - hydroxyphenyl)acetic acid
- 4 - (2 - propenyl)phenol
- 4 - phenylphenol
- 10 4 - (3 - bromophenyl)phenol
- 4 - benzylphenol
- 4 - (3 - fluoro - 2 - propenyl)phenol
- 4 - (4 - chlorobenzyl)phenol
- 4 - (3 - ethylphenyl)phenol
- 15 4 - (2 - chloro - 3 - ethylphenyl)phenol
- 2,5 - xlenol

Primary oxidation dye precursors can be used alone or in combination with other primary oxidation dye precursors of the same type (e.g., diamines with other diamines and polyhydric phenols with other polyhydric phenols), and one or more primary oxidation dye precursors of the same type can be used in combination with one or more secondary oxidation dye precursors of the same type (e.g., a combination of two primary oxidation dye precursors of the diamine type can be used in combination with a secondary oxidation dye precursor of the amine type). Oxidation dye precursors of the phenolic or polyhydric phenol type should not be mixed with dye precursors of the diamine or amine type. The choice of a single oxidation dye precursor or of a particular combination of oxidation dye precursors will be determined by the color, shade and intensity of coloration which is desired. The total concentration of dye precursor in the coloring solution can be within the range from 0.001% to 6% by weight and is preferably from 0.01% to 1.0% by weight.

The oxidizing agent for use in the process herein is oxygen. The process is most easily carried out by simply exposing the coloring solution, containing the substrate to be colored, to the atmosphere. However, any other means by which the solution is brought into contact with oxygen can be used. For example, oxygen gas under pressure can be bubbled through the coloring solution, or hydrogen peroxide and an enzyme such as catalase, which produces oxygen from hydrogen peroxide, can be introduced into the coloring solution.

The coloring process of the present invention is preferably carried out in aqueous solution (the term solution herein, also encompasses fine dispersions and colloids of the reacting and reacted materials) but any liquid solvent medium which does not interfere with the coloring process can be used. Examples of alternative solvents are glycerol, methanol and formamide. The time of exposure of the substrate to the coloring solution

can be within the range from 30 seconds to one or more hours, but is preferably from 2 to 10 minutes.

It is also recognized that conventional hair coloring products contain thickeners such as carboxymethylcellulose, surfactants such as sodium N-laurylsarcosinate and conditioners such as solubilized lanoline derivatives, and that such ingredients may also be utilized in the coloring process of the present invention when dyeing hair.

In a preferred manner of carrying out the process of the present invention, an aqueous solution is prepared containing from 0.01% to 1.0% by weight of one or more primary oxidation dye precursors and optionally one or more secondary oxidation dye precursors. If a combination of oxidation dye precursors is used they must be of the same type, as described hereinbefore. The pH of the solution is adjusted and maintained throughout the process within the range from 5.5 to 8.0 by a suitable means, such as by buffering with suitable salts (e.g., a mixture of  $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$ ) or by adding acid or base as required. The oxidase enzyme is then added in an amount such that the concentration of enzyme in the solution is from 0.05 to 100 ppm. The hair to be colored is immersed in the solution. Preferably the hair should be immersed in the solution before the addition of enzyme or as soon as practical thereafter, so that as color bodies form in the solution they will become quickly affixed to the hair. After the hair has reached the desired color (normally in 2 to 10 minutes) it is removed from the solution, rinsed and dried.

For convenience, the oxidase enzymes and oxidation dye precursors can be formulated together in a composition which can be diluted to the proper usage-concentration in solution immediately prior to use. These compositions are preferably powdered compositions, since if the enzyme and primary oxidation dye precursors are in solution, and any oxygen is present, premature conversion of the oxidation dye precursor to the corresponding dye will take place. The compositions of the invention comprise the enzyme and one or more primary, and optionally one or more secondary, oxidation dye precursors in a ratio within the range from 6,000,000:1 to 0.02:1, preferably from 20,000:1 to 1:1. If a combination of oxidation dye precursors is used, they must be of the same type, as described hereinbefore. These compositions can be added directly to an aqueous buffer solution in contact with oxygen immediately before use. Although the compositions may contain only enzyme and oxidation dye precursor, they normally contain these materials in combination with an inert diluent to facilitate handling and measuring. The diluents are preferably powdered solids such as inorganic

salts (e.g., sodium chloride and calcium sulfate) starches and sugars. Normally, the total amount of enzyme and oxidation dye precursor in the compositions will be within the range from 0.5% to 20% by weight, the remainder of the composition being the diluent materials and optionally other materials such as hair conditioners, and surfactants.

The following examples illustrate the invention. All enzyme concentrations referred to in these examples are based on weight of pure enzyme.

#### Example I

This example is to illustrate the color formation test with various suitable primary oxidation dye precursors, and the dyeing effect which these precursors have on human hair when used according to the process of the invention. The hair used was medium blond virgin European human hair which was formed into approximately 2 gram switches, by dipping one end of the hair into about 0.25 inches of glue.

Solutions were prepared comprising 10 ml of aqueous  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer (0.1 M in phosphate, pH 6) and 0.1 to 1 ml of a 1% (by weight) aqueous or alcoholic solution of primary dye precursor. The solutions also contained 0.3% of  $\text{H}_2\text{O}_2$  and 1 ppm of catalase to insure saturation of the solution with  $\text{O}_2$ , although this is not required as long as the solution is in contact with air. 50 or 100  $\mu\text{l}$  of a 1 mg/ml aqueous solution of lac-case enzyme was then added. The solution was allowed to stand exposed to the air, and color formation occurred within 20 minutes. When hair was to be dyed, the solutions were prepared in the same manner, except that a human hair switch was added to the solution before the addition of enzyme, and the hair was stirred with a glass rod in the solution for 10 to 20 minutes after the addition of enzyme to obtain even dyeing. After dyeing, the hair was rinsed, patted with a towel and allowed to dry. Results are shown in the following table.

TABLE 2

Precursor	Color Formation			Hair Dyeing		
	Dye Precursor Conc.	Solution Color Before Enzyme	Solution Color After Enzyme	Dye Precursor Conc.	Initial Hair Color	Final Hair Color
o-Phenylenediamine	0.1%	pale yellow	orange	0.1%	medium blond	yellow-orange
p-Phenylenediamine	0.1%	clear	red-brown	0.1%	medium blond	medium-brown-black
N,N-dimethylphenyl-enediamine	0.1%	pale red	bright red	0.1%	medium blond	blond with auburn highlights
N-Phenyl-p-phenyl-enediamine	0.1%	pale blue	yellow-brown	0.1%	medium blond	medium brown
3-methoxy-4-hydroxy benzaldehyde*	0.1%	clear	pale-brown	0.1%	medium blond	blond with brown highlights
2,4-Toluenediamine*	0.1%	clear	pale-brown	0.1%	medium blond	blond with brown highlights

\*Indicates 100  $\mu$ l laccase solution used. 50  $\mu$ l was used with the other precursors in the table.



- Substantially similar results to those above were obtained when the laccase enzyme was replaced by an equal weight amount of any one of the following oxidase enzymes: lactate oxidase, glucose oxidase, galactose oxidase, L-2 hydroxyacid oxidase, monoamine oxidase, urate oxidase and aldehyde oxidase.
- Example II  
This example illustrates the results obtained with additional suitable primary oxidation dye precursors in the color formation test of Example I, using laccase as the oxidase enzyme.

TABLE 3

## Color Formation Test

Precursor	Dye Precursor Conc.	Solution Color Before Enzyme	Solution Color After Enzyme
o-hydroxyphenol	0.1%	clear	medium brown
2-methoxyphenol	0.1%	pale yellow	pale orange
3,4-Dihydroxybenzaldehyde*	0.1%	clear	yellow-red
4-Hydroxy-3-methoxy cinnamic acid	0.1%	clear	pale yellow
4-methoxyphenol	0.1%	clear	pale yellow

\*Indicates 100  $\mu$ l laccase solution used. 50  $\mu$ l was used with the other precursors in the table.

- Example III  
This example illustrates the performance of the secondary dye precursor test on certain aromatic amines, phenols, and derivatives thereof which do not give a color formation reaction in the primary dye precursor test. Pairs of solutions were prepared comprising 9 ml of aqueous  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer (0.1 M in phosphate, pH 6) and 1 ml of a 1% by weight aqueous or alcoholic solution of primary dye precursor. The solutions also contained 0.3%  $\text{H}_2\text{O}_2$  and 1 ppm catalase to insure saturation of the solution with oxygen, although this is not required. 1 ml of a 1% by weight solution of a secondary dye precursor was then placed in one of the solutions and 100 or 200  $\mu$ l of laccase solution was added to both solutions. The color of the pair of solutions was compared after standing for 5 minutes in contact with air. Results are shown in Table 4.

TABLE 4  
Color Formation Test — Secondary Dye Precursors

Primary Dye Precursor	Conc.	Secondary Dye Precursor	Conc.	Solution Color Primary Only	Solution Color Primary & Secondary
p-phenylenediamine	0.1%	p-chloroaniline	0.1%	purple	dark yellow
p-phenylenediamine	0.1%	4-methylaniline	0.1%	purple	medium burgundy
p-phenylenediamine	0.1%	aniline	0.1%	purple	blue-black
o-phenylenediamine	0.1%	aniline	0.1%	orange	yellow-orange
3-methoxy-4-hydroxy benzaldehyde*	0.1%	2,5-xylenol	0.1%	pale yellow	cloudy yellow
3-methoxy-4-hydroxy benzaldehyde*	0.1%	2,4-dihydroxy-benzaldehyde	0.1%	pale yellow	pale green
3-methoxy-4-hydroxy benzaldehyde*	0.1%	p-hydroxy-benzaldehyde	0.1%	pale yellow	pale green

\*200  $\mu$ l laccase enzyme (1 mg/ml stock solution) was used in the tests involving 3-methoxy-4-hydroxy benzaldehyde, 100  $\mu$ l in the others.

When in this test p-fluoroaniline was substituted on an equal weight basis for the amino-containing secondary oxidation dye precursors in Table 4, substantially similar results were obtained in that the color, shade or intensity of color produced in solution by o-phenylenediamine and p-phenylenediamine was modified.

Likewise, when in this test the following primary oxidation dye precursors were substituted on an equal weight basis for o-phenylenediamine and p-phenylenediamine, substantially similar results were obtained in that the color, shade or intensity of color of the primary oxidation dye precursor was modified by the amino-containing secondary oxidation dye precursors of Table 4: 2,4-toluenediamine, N-phenyl-p-phenylenediamine, and N,N-dimethyl-p-phenylenediamine.

When in this test the following polyhydric phenols and derivatives thereof, which are suitable primary oxidation dye precursors, were substituted for 3-methoxy-4-hydroxy benzaldehyde, substantially similar results were obtained in that the color, shade or intensity of color of the primary oxidation dye precursor was modified by the hydroxyl-containing secondary dye precursors: o-hydroxyphenol, 3,4-dihydroxybenzaldehyde, 2-methoxyphenol, 4-hydroxy-3-methoxy cinnamic acid and p-methoxyphenol.

oxyphenol, 4-hydroxy-3-methoxy cinnamic acid and p-methoxyphenol.

#### Example IV

This example illustrates the effect which secondary oxidation dye precursors have on the color produced by primary oxidation dye precursors in dyeing hair. Switches of human hair were prepared as in Example I except that the hair was bleached blond with some residual yellow in it.

Dyed switches, to be used as standards for color comparison, were prepared with primary oxidation dye precursors as follows. Solutions were prepared comprising 9 ml. of aqueous  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer (0.1 M in phosphate, pH 6) and 1 ml. of a 1% by weight aqueous or alcoholic solution of primary oxidation dye precursor. The solutions also contained 0.3%  $\text{H}_2\text{O}_2$  and 1 ppm catalase to insure saturation of the solution with oxygen, although this is not required. Switches of hair were placed in the solutions, laccase enzyme was added, and the solutions were stirred for 10 minutes in contact with air. The hair was then removed from the solutions, rinsed in running tap water, patted with a towel and allowed to dry. Results were as follows:

TABLE 5

Primary Dye Precursor	Conc.	Color Developed On Hair	Standard Switch #
p-phenylenediamine	0.1%	dark steel gray	(1)
3-methoxy-4-hydroxy benzaldehyde*	0.1%	blond with gold highlights	(2)

\*200  $\mu\text{l}$  laccase (1 mg/ml stock solution) was used with 3-methoxy-4-hydroxy benzaldehyde, 100  $\mu\text{l}$  with p-phenylenediamine.

To evaluate the secondary oxidation dye precursors, the hair dyeing process was carried out as above except that 1 ml. of a 1% by weight aqueous or alcoholic solution of secondary oxidation dye precursor was added to the solution after the primary oxidation dye

precursor was added, but before laccase was added. After the dyed hair was dried, its color was compared to the appropriate standard switch dyed only with the primary oxidation dye precursor. Comparative results are shown in Table 6.

TABLE 6

Primary Dye Precursor	Conc.	Secondary Dye Precursor	Conc.	Color Developed On Hair	Compare to Standard Switch #
p-phenylenediamine	0.1%	aniline	0.1%	medium purple- brown	(1)
p-phenylenediamine	0.1%	p-chloroaniline	0.1%	brown-auburn	(1)
p-phenylenediamine	0.1%	p-toluidine	0.1%	brown-purple	(1)
3-methoxy-4-hydroxy benzaldehyde*	0.1%	2,4-dihydroxy- benzaldehyde	0.1%	blond-green highlights	(2)
3-methoxy-4-hydroxy benzaldehyde*	0.1%	p-hydroxybenzal- dehyde	0.1%	blond-green highlights	(2)

\*200  $\mu$ l laccase enzyme (1 mg/ml stock solution) was used in the tests involving 3-methoxy-4-hydroxy benzaldehyde, 100  $\mu$ l in the tests involving p-phenylenediamine.

This example illustrates the coloring of human hair with a composition of the present invention. All percentages are by weight. 120 grams of a pH 6  $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$  aqueous buffer (0.1 M in phosphate) were prepared, containing 2.5% of ethanol (solvent and wetting agent) 0.1% of Dow Corning EF. 13574A (a cationic polysiloxane conditioner from the Dow Corning Company), 5% of Culveram CDG (a betaine-type surfactant from the Culver Chemical Company), and 0.5% of JR-IL (a cationic cellulose derivative thickener from Union Carbide Company). 60 grams of a composition of the invention were prepared consisting of 1.5% of o-phenylenediamine, 0.0075% of laccase and 98.4925% of sodium sulfate. The 60 grams of composition were mixed together with the 120 grams of buffer, and the mixture was immediately applied to the hair of a human female subject having naturally light brown hair, by working it into a rich lather which remained on the hair and did not run down the neck and forehead. After working the mixture for 2 minutes (to insure uniform application to all of the hair) the foamy lather was allowed to remain on the hair an additional 5 minutes. The subject then rinsed her hair thoroughly with tap water and allowed it to dry. It was observed that the hair had changed from its original light brown color to a medium auburn shade.

Hair coloring products employing oxidation hair dyes are typically marketed in kit form, i.e. a package comprising an individually packaged oxidizing component and an individually packaged oxidation dyeing component. In an embodiment of this invention, the oxidizing component comprises a packet containing an oxidase enzyme as hereinabove detailed, the oxidation dyeing component comprises one or more primary oxidation dye precursors of the type hereinbefore detailed and optionally, one or more secondary oxidation dye precursors, the amounts of these components being such that when the total contents of the packets are dissolved in water as directed on the package, the resulting solution will contain from 0.01 to 500 ppm enzyme and from 0.001% to 6% by weight of dye precursor. The components are mixed with water by the user immediately prior to application to the hair in the presence of oxygen (air). An example of such kit is as follows:

A hair dyeing kit was assembled comprising a single package including therein: (1) a foil packet containing an oxidation dyeing component, said component consisting of 4 g. of p-phenylenediamine, 2 g. of sodium carboxymethylcellulose and 20 g. of starch; and (2) a packet containing 0.01 gr. of laccase and 3 g. of  $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$  (pH 7 buffer). The oxidation dyeing component was admixed with 120 ml. of water, the enzyme component

was added and the solution applied to the hair in the presence of air and provided a brown-black color, which was substantially shampoo-stable.

In the above kit, the laccase was replaced by an equivalent amount of lactate oxidase, glucose oxidase, galactase oxidase, L-2 hydroxyacid oxidase, aldehyde oxidase, monoamine oxidase and urate oxidase, respectively, and equivalent results were secured.

In the above kit, the p-phenylenediamine was replaced by an equivalent amount of o-phenylenediamine, 2,4-toluenediamine, N-phenyl - p - phenylenediamine, N,N - dimethyl - p - phenylenediamine, 2 - nitro - o-phenylenediamine, o - hydroxyphenol, p-hydroxyphenol, 3,4 - dihydroxybenzaldehyde, 2 - methoxy - 4 - (1 - propenyl)phenol, 4-methoxyphenol, p - methoxyaniline, N-methyl - p - phenylene diamine, 4 - hydroxy-3 - methoxycinnamic and mixtures thereof, respectively, said mixtures not containing amines in combination with phenols, and shampoo-fast hair colors were secured.

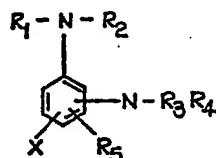
#### WHAT WE CLAIM IS:—

1. A process for colouring hair which comprises contacting the hair with a solution containing within the range from 0.01 ppm to 500 ppm of an oxidase enzyme selected from laccase, lactate oxidase, glucose oxidase, galactose oxidase, L-2 hydroxyacid oxidase, aldehyde oxidase, monoamine oxidase and urate oxidase, and from 0.001% to 6% by weight of an aromatic compound which is a primary oxidation dye precursor (as hereinbefore defined), in the presence of oxygen, the solution having a pH within the range from 4 to 10, and being free from mixtures of aromatic amines or derivatives thereof with polyhydric phenols or derivatives thereof.
2. A process as claimed in claim 1, wherein the concentration of oxidase enzyme is within the range from 0.05 ppm to 100 ppm.
3. A process as claimed in claim 1 or 2, wherein the hair to be coloured is contacted with the solution containing the primary oxidation dye precursor prior to the addition of the enzyme, and the hair remains in contact with the solution after the enzyme has been added.
4. A process as claimed in any one of claims 1 to 3, wherein the concentration of primary oxidation dye precursor is within the range from 0.01% to 1.0% by weight.
5. A process as claimed in any one of claims 1 to 4, wherein the pH is within the range from 5.5 to 8.
6. A process as claimed in any one of claims 1 to 5, wherein the solution additionally contains a secondary oxidation dye precursor (as hereinbefore defined) and the total concentration of primary and secondary oxidation dye precursors is within the range from 0.001% to 6% by weight.

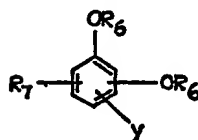
7. A process as claimed in claim 6, wherein the total concentration of primary and secondary oxidation dye precursor is within the range from 0.01% to 1.0% by weight.

5 8. A process as claimed in any one of claims 1 to 7, wherein the solution used for contacting the hair is an aqueous solution.

9. A process as claimed in any one of claims 1 to 8, wherein the primary oxidation dye precursor is a compound of the formula:



or



wherein X is hydrogen, halogen, nitro, amino,



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—COOM or —SO<sub>3</sub>M (where M is hydrogen, alkali metal, alkaline earth metal, ammonium or substituted ammonium in which one or more hydrogens on the ammonium ion is replaced with an alkyl containing from 1 to 3 carbon atoms), Y is hydrogen, halogen, nitro, hydroxyl,



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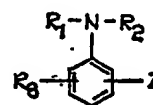
—COOM', or SO<sub>3</sub>M' (where M' is hydrogen, alkali metal or alkaline earth metal), R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub>, which may be the same or different, are each hydrogen, a C<sub>1</sub>—C<sub>4</sub> alkyl or alkenyl or a C<sub>6</sub> to C<sub>8</sub> aryl, alkaryl or alkaryl and R<sub>5</sub> is hydrogen, a C<sub>1</sub> to C<sub>4</sub> alkyl or alkenyl (including substituted alkyl or alkenyl where the substituents are the same as those designated X above), or a C<sub>6</sub> to C<sub>8</sub> aryl, alkaryl or alkaryl (including substituted aryl, alkaryl or alkaryl where the substituents are selected from those designated X above), R<sub>6</sub> is hydrogen, a C<sub>1</sub>—C<sub>4</sub> alkyl or alkenyl (including substituted alkyl or alkenyl where the substituents are the same as those designated Y above) and R<sub>7</sub> is hydrogen, a C<sub>1</sub>—C<sub>4</sub> alkyl or alkenyl (including substituted alkyl or alkenyl in which the

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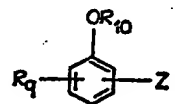
substituents are the same as those designated Y above) or C<sub>6</sub> to C<sub>8</sub> aryl, alkaryl or alkaryl group (including substituted aryl, alkaryl or alkaryl in which the substituents are the same as those designated Y above).

10. A process as claimed in claim 9, wherein the primary oxidation dye precursor is: *p*-phenylenediamine, *o*-phenylenediamine, 2,4-toluene diamine, N,N-dimethyl-*p*-phenylenediamine, N-phenyl-*p*-phenylenediamine, and mixtures thereof, and 3-methoxy-4-hydroxybenzaldehyde, *o*-hydroxy-phenol, 2-methoxyphenol, 3,4-dihydroxybenzaldehyde, 4-hydroxy-3-methoxy cinnamic acid, 4-methoxyphenol or mixtures thereof.

11. A process as claimed in any one of claims 6 to 10, wherein the secondary oxidation dye precursor is a compound of the formula:



or



where R<sub>1</sub> and R<sub>2</sub> are as defined in claim 9, Z is hydrogen, C<sub>1</sub> to C<sub>4</sub> alkyl, halogen, nitro,



—COOM or —SO<sub>3</sub>M (where M has the meaning set out in claim 9) and Z' is hydrogen, a C<sub>1</sub> to C<sub>4</sub> alkyl, halogen, nitro,



—COOM' or SO<sub>3</sub>M' (where M' has the meaning given in claim 9), R<sub>3</sub> is hydrogen, a C<sub>1</sub> to C<sub>4</sub> alkyl or alkenyl (including substituted alkyl or alkenyl wherein the substituents are selected from those designated as Z, above) or a C<sub>6</sub> to C<sub>8</sub> aryl, alkaryl or alkaryl group (including substituted aryl, alkaryl or alkaryl wherein the substituents are selected from those defined as Z, above), R<sub>4</sub> is hydrogen, a C<sub>1</sub> to C<sub>4</sub> alkyl or alkenyl (including substituted alkyl or alkenyl in which the substituents are selected from those designated Z' above) or C<sub>6</sub> to C<sub>8</sub> aryl, alkaryl or alkaryl (including substituted aryl, alkaryl or alkaryl wherein the

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substituents are selected from those designated Z' above), and R<sub>10</sub> is hydrogen, a C<sub>1</sub> to C<sub>4</sub> alkyl or alkenyl (including substituted alkyl or alkenyl in which the substituents are selected from those designated Z above).

12. A process as claimed in claim 11, wherein the secondary dye precursor is: p-chloroaniline, p-fluoroaniline, aniline, 4-methylaniline, and mixtures thereof and 2,5-xyleneol, 2,4-dihydroxybenzaldehyde, p-hydroxybenzaldehyde and mixtures thereof.

13. A process for colouring hair substantially as herein described with reference to Examples I, IV and V.

14. A composition for colouring hair, which comprises, in admixture, an oxidase enzyme selected from laccase, lactate oxidase, glucose oxidase, galactose oxidase, L-2 hydroxyacid oxidase, aldehyde oxidase, monoamine oxidase and urate oxidase, and one or more primary oxidation dye precursors (as hereinbefore defined), optionally together with one or more secondary oxidation dye precursors (as hereinbefore defined), and optionally an inert diluent, the ratio of the enzyme present to the dye precursors present being within the range from 6,000,000:1 to 0.02:1.

15. A composition as claimed in claim 14, wherein the ratio of enzyme to dye precursors present is within the range from 20,000:1 to 1:1.

16. A composition as claimed in claim 14 or 15, wherein the composition is in powdered form and contains a powdered solid as inert diluent.

17. A composition as claimed in any one of claims 14 to 16, wherein the total amount of enzyme and oxidation dye precursors present is within the range from 0.5% to 20%, by weight, the remainder of the composition being inert diluent and optionally, other suitable additives.

18. A hair colouring kit adapted to be used in the process of claim 1, comprising an individual oxidation dye component which is a primary oxidation dye precursor (as hereinbefore defined), optionally in admixture with a secondary oxidation dye precursor (as hereinbefore defined) and an individually packaged oxidizing component which is an enzyme selected from: laccase, lactate, oxidase, glucose oxidase, galactose oxidase, L-2 hydroxy acid oxidase, aldehyde oxidase, monoamine oxidase, and urate oxidase, the ratio of the amount of oxidation dye component to the amount of oxidizing component being such that when the whole contents of the kit are dissolved in an appropriate amount of solvent, the resulting solution contains from 0.001% to 6% by weight of oxidation dye precursor and from 0.01 ppm to 500 ppm of enzyme and has a pH from 4 to 10 and is free from mixtures of aromatic amines or derivatives thereof with polyhydric phenols, or derivatives thereof.

19. A hair colouring kit as claimed in claim 18, wherein the primary oxidation dye precursor is selected from: p - phenylene diamine, o - phenylene diamine, 2,4 - toluene diamine, N - phenyl - p - phenylene diamine, 2 - nitro - p - phenylene diamine, N - methyl - p - phenylene diamine, 4 - nitro - o - phenylene diamine, o - hydroxyphenol, p - hydroxyphenol, 3,4 - dihydroxybenzaldehyde, 2-methoxy - 4 - (1 - propenyl)phenol, 4 - methoxyphenol, p - methoxyaniline and 4-hydroxy - 3 - methoxycinnamic acid.

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